Atherosclerosis 269 (2018) 144–150

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Antithrombin levels are associated with the risk of first and recurrent arterial thromboembolism at a young age

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ARTICLE INFO

Article history:
Received 21 July 2017
Received in revised form
29 November 2017
Accepted 12 January 2018
Available online 16 January 2018

ABSTRACT

Background and aims: It is as yet unknown whether antithrombin levels are associated with arterial thromboembolism (ATE) at a young age. To investigate the association between antithrombin levels and premature and recurrent ATE, we performed a case-control study and a subsequent nested cohort study of premature coronary heart disease (CHD) patients.

Methods: In the case-control study, we included 571 patients who had a recent premature ATE, including CHD and ischemic stroke (IS), and 461 healthy controls. The association between antithrombin levels (dichotomized: ≤median *vs.* >median) and ATE was investigated. Subsequently we studied the association between antithrombin levels and recurrent cardiac events, ATE or death in a nested cohort of 323 CHD patients.

Results: Low antithrombin levels (≤median, 1.04 IU/mL) are associated with an increased risk of ATE (OR 1.46; 95% CI:1.09–1.96), after adjustment for classical cardiovascular risk factors. This was observed in the subgroups of CHD patients (1.43; 1.01–2.02) and IS patients (1.48; 1.01–2.19). CHD patients with low antithrombin levels had a higher risk of recurrent cardiac events (HR 2.16, 95% CI:1.07–4.38). Especially in women with low antithrombin levels, the risk of recurrent cardiac events was high (HR 5.97, 95% CI 1.31–27.13) as was the risk of recurrent ATE or death (HR 4.22, 95% CI 1.19–15.00).

Conclusions: Individuals with relatively low antithrombin levels have an increased risk for ATE at a younger age. CHD patients with low antithrombin levels, especially women, have a higher risk of recurrent cardiac events.

1. Introduction

Antithrombin is a strong inhibitor of blood coagulation through inactivation of mainly thrombin and factor Xa. The anticoagulant function of antithrombin *in vivo* is thought to be activated by heparan sulphate on the vascular endothelium [1]. It has been suggested that strongly reduced antithrombin levels as in hereditary antithrombin deficiency (around 50% of normal) are associated with arterial thrombotic events (ATE) [2]. Furthermore, a pooled analysis of four cohort studies showed that inherited thrombophilia, including antithrombin deficiency, increased the risk of ATE more pronouncedly in women than in men [3]. However, it is as yet unknown whether slightly reduced levels are associated with ATE. The process of atherosclerosis is driven by traditional cardiovascular risk factors, and the occurrence of ATE is typically caused by rupture of atherosclerotic lesions and subsequent thrombus formation. A lack of inhibition of thrombus formation, for instance by low antithrombin levels, may cause acceleration of thrombus formation and increased risk of occlusion of blood vessels. This may cause an increased risk of ATE [3]. Hemostatic factors are thought to play a more important pathophysiological role in ATE in young patients than in older patients because in young individuals atherosclerosis is not as extensive as in elderly. In addition, in young patients with myocardial infarction, normal coronary arteries are more often found than in older patients [4–6]. Indeed, levels of hemostatic factors such as fibrinogen, TAFI and ADAMTS13 have been associated with cardiovascular disease at a young age [7–9].

The mortality after myocardial infarction in young patients has

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https://doi.org/10.1016/j.atherosclerosis.2018.01.014

been reported as high as 8% at 8 years after percutaneous coronary interventions [10]. Risk factors for mortality after a myocardial infarction include heart failure, ventricular arrhythmias, angina pectoris and re-infarction [11]. Finding more risk factors may allow for a more precise identification of patients at risk for recurrence or death in those with documented coronary heart disease. We hypothesize that low-normal antithrombin levels are associated with both a first and recurrent ATE at a young age, and may differ between sexes, as this has been shown before in other thrombophilias [3]. Therefore, we investigated the association of antithrombin levels with the risk of first and recurrent ATE at a young age, including a sex-specific analysis.

2. Patients and methods

2.1. Patients

We performed two related studies: first we performed a case-control study, and subsequently, we performed a follow-up (cohort-) study of the patients with coronary heart disease from the first study.

2.2. Case-control study

The 'Genetic risk factors for Arterial Thrombosis at a young age: the role of TAFI and other Coagulation factors (ATTAC)' study is a single-center, case-control study investigating the role of coagulation factors on incidence of ATE at a young age. The design of the study and recruitment of patients and controls have been reported elsewhere in detail [7,8]. In short, cases were consecutive patients who presented with a first acute arterial ischemic event in our center. Our center is a university hospital with a community function, located in the city centre. Our center employs no selection criteria of admission of cardiovascular patients, but young patients are over-represented. Patients were men aged 45 years or younger or women aged 55 or younger. The case-control study originally consisted of three subgroups: Group CHD: coronary heart disease, including acute myocardial infarction and unstable angina pectoris (CHD); group IS: ischemic stroke (IS) or transient ischemic attack (TIA); and group PAD: peripheral arterial disease (PAD). Patients were included 1–3 months after the event, to avoid a possible acute phase response on plasma levels of the parameters investigated. Controls were neighbours or friends of the patients. They fulfilled the same age criteria but did not have a history of ATE.

2.3. Follow-up study

The 368 subjects included in the ATTAC study who presented with coronary heart disease were asked to participate in a follow-up study as reported elsewhere in more detail [9]. These subjects were followed at the outpatient clinic (and by telephone in case this was not possible) for recurrent cardiac events and for any recurrent cardiovascular event or all-cause mortality.

Participants were included in the study after written informed consent, between October 2001 and June 2010. Both the ATTAC and the follow-up studies were approved by the institutional Medical Ethics Committee of the Erasmus MC, and both conform to the ethical guidelines of the 1975 declaration of Helsinki.

2.4. Definitions

The definitions used in this study have been reported elsewhere [8]. In short, CHD was defined as acute myocardial infarction (AMI) or unstable angina pectoris (UAP). AMI was defined as typical chest pain, with elevated cardiac markers (CK MB/troponin T), and/or characteristic electrocardiographic findings. UAP was defined as typical chest pain at rest. Transient ischemic attack (TIA) was defined as the acute onset of focal cerebral dysfunction, which could not be explained otherwise than by focal cerebral ischemia, as diagnosed by a neurologist. Symptoms had to be temporary and last less than 24 h. Ischemic stroke (IS) was defined as the acute onset of focal cerebral dysfunction as a result of cerebral ischemia, with symptoms lasting longer than 24 h. Brain imaging by CT-scan and MRI-scan had to be compatible with the diagnosis and were used to rule out intracerebral hemorrhage. Smoking status was defined as self-reported never, previous or current smoker. Hypercholesterolemia was originally defined as a subject-reported presence of hypercholesterolemia or the use of lipid lowering treatment on the day of the event. As most cases used cholesterol lowering medications at inclusion, cholesterol measurements were not representative of cholesterol levels before the event. Therefore, in the case-control study, cholesterol was not adjusted for in the analyses. In the follow-up study, as nearly all patients used lipid-lowering treatment, cholesterol levels were adjusted for in the analyses. Patients with a medical history of diabetes or using either oral anti-diabetic medication or insulin therapy on the day of the event were considered to have diabetes. Hypertension was defined by a systolic blood pressure $\geq$140 mmHg, and/or diastolic blood pressure $\geq$90 mmHg or the use of anti-hypertensive drugs.

In the follow-up study we used the endpoints recurrent cardiac event (defined as myocardial infarction or revascularization procedure) and any recurrent arterial thrombotic event or death (defined recurrent cardiac event, cerebrovascular event (IS or TIA) or all-cause mortality). At the time of inclusion in the study, direct acting oral anticoagulants were not yet registered in the Netherlands.

2.5. Blood sampling

Blood was collected 1–3 months after the event in sodium citrate (final concentration 0.105M) using a Vacutainer System (Beckton, Dickinson and Company, Plymouth, UK) and centrifuged at 2000*g* for 10 min at 4 °C. Plasma was additionally centrifuged for 10 min at 20,000*g* for 10 min at 4 °C and stored in aliquots at −80 °C. Technicians were not aware of the case–control status of the samples. Antithrombin activity measurements were performed using the factor Xa-based INNOVANCE® Antithrombin assay kit (Siemens). Measurements of samples were performed once, but AT values outside the reference range were performed in duplicate. Cholesterol and HDL were determined on Modular Analytics® (Roche Diagnostics, Mannheim, Germany).

2.6. Statistical analysis

Descriptive statistics were used in both studies. The data are presented as means ± standard deviation.

2.6.1. Case-control study

To compare levels of the normally distributed risk factors between groups, ANOVA's were performed with adjustment for age and sex. We performed logistic regression for ATE with antithrombin activity as a continuous variable and as a binominal variable, using the predefined cut-off-levels of the lower limit of the reference range ($\leq$0.80 U/mL) and the median antithrombin level of controls. Logistic regression analyses were adjusted for age, sex, BMI, smoking, family history, hypertension, diabetes mellitus. In addition, separate sex-specific analyses were performed, adjusted for the same cardiovascular risk factors.

2.6.2. Follow-up study

In the follow-up study, cumulative event-free survival curves were constructed using the Kaplan-Meier method. Comparisons in the Kaplan-Meier curves were performed using the Log-rank test. The median antithrombin level of the cohort of CHD patients was the predefined cut-off level. To determine by how much low antithrombin levels in this cohort increase the risk of ATE, Cox-regression analysis was performed with the median antithrombin levels after adjustment for age, BMI and sex, and after adjustment for age, BMI and sex, family history, cholesterol, HDL, hypertension, diabetes, and smoking (at inclusion).

All statistical analyses were performed using SPSS for Windows, version 21 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Case-control study

The ATTAC study included a total of 1157 participants. For 76 participants (38 cases, 38 controls), plasma was no longer available, and 49 were PAD patients. This current study, therefore, included 1032 individuals, of whom 571 CHD and IS cases and 461 healthy controls. Forty-five percent of cases (257/620) and thirty-five percent of controls (162/461) were men. The mean age was 39.8, standard deviation (SD) ±7.8 for cases and 43.0 SD ± 6.8 for controls. As expected, traditional risk factors were more prevalent in cases than in controls (Table 1).

When investigating classical risk factors for ATE using logistic regression, we found that age (OR 1.05, 95% CI:1.03–1.07), male sex (OR 2.08, 95% CI: 1.54–2.83), family history of cardiovascular disease (OR 2.25, 95% CI:1.66–3.05), hypertension (OR 3.98, 95% CI:2.54–6.24), and diabetes (OR 4.90, 95% CI: 2.17–10.89) were significantly associated with ATE. Hypercholesterolemia was associated with a lower risk of ATE (OR 0.65, 95% CI:0.43–0.96).

The median of antithrombin activity was similar across controls and subtypes of cases (in controls, CHD and IS: 1.04 U/mL). The distribution of antithrombin levels in the three groups is shown in Fig. 1. Since the distribution approached a normal distribution (even though the Kolmogorov-Smirnov test did not support a normal distribution), we performed parametric statistics, since this enables us to adjust for confounders and effect modifiers. Ten participants had antithrombin values ≤ 0.80 U/mL (lower limit of reference range). Logistic regression without adjustment for classical risk factors showed no predictive value of antithrombin activity as a continuous variable (OR1.53; 95% CI:0.48–4.89) or at the cut-offs of 0.80 U/ml (OR 1.21; 95% CI:0.34–4.33) and median antithrombin level (≤1,04 U/ml) (OR 1.12; 95% CI:0.87–1.43). However, when using antithrombin activity as a binary variable with predefined cut-off of the median antithrombin level, a 46% increased risk of ATE was found in the adjusted analysis for the participants with low antithrombin levels (OR 1.46; 95% CI:1.09–1.96). Similar results were found in the CHD group (OR 1.43; 95% CI:1.01–2.02) and in the IS group (OR 1.48; 95% CI: 1.01–2.19). In a separate sex-specific analysis, no major differences were found between men and women. To investigate if the higher age at inclusion for women influenced results, women with the age of 46 or older were excluded. The adjusted OR was 1.55, 95% CI: 1.09–2.21. These results are summarized in Table 2.

3.2. Follow-up study

Three-hundred-fifty-three subjects with coronary heart disease entered the ATTAC follow-up study, of whom only 323 subjects could be evaluated, because no blood samples were available from 30 patients. Those subjects consisted of 181 men and 142 women, characteristics are summarized in Supplemental Table 1. At study entry, the median age was 42 years for men and 47 years for women. Seventy-one percent had single vessel disease. Most patients (94%) received a stent: 9% a bare metal stent, 85% a drug-eluting stent. No coronary artery bypass surgery was performed.

Table 1
Patient characteristics of the ATTAC case-control study on the risk of a first arterial thromboembolism.

	Controls (n = 461)	Cases (n = 571)	*p*-value
Demographics			
Age, mean (years)	39.8 ± 7.8	43.0 ± 6.8	<0.01
Men, n (%)	162 (35.1)	257 (45.0)	<0.01
Body mass index, mean (kg/m^2 ±SD)	25.7 ± 4.5	27.0 ± 4.7	<0.01
Index event			
UAP + AMI (n)		368	
Stroke + TIA (n)		203	
Risk factors			
Family history of ATE (%)	22.6	51.5	
Smoking (former + current) (%)	52.5	75.7	<0.01
Hypertension (%)	6.5	28.2	0.12
Systolic blood pressure, mmHg, median ±SD	123 ± 16	122 ± 21	
Diastolic blood pressure, mmHg, median ±SD	80 ± 11	80 ± 13	
Diabetes (%)	1.7	10.2	<0.01
Cholesterol, mean, ±SD	5.17 ± 0.94	4.29 ± 0.96	<0.01
LDL, mean, ±SD	3.17 ± 0.89	2.51 ± 0.85	<0.01
HDL, mean, ±SD	1.52 ± 0.43	1.29 ± 0.42	<0.01
Antithrombin activity (U/mL, SD)	1.04 ± 0.10	1.04 ± 0.11	0.77
Antithrombin activity ≤0.80 U/mL	4	6	0.47
Medication at study entry			
Platelet function inhibitor	5	541	
Vitamin K antagonist	4	47	
Beta-blocker	14	229	
Ace-inhibitor or A2 antagonist	12	293	
Calcium blocker	1	48	
Diuretic	7	72	
Statin or fibrate	9	483	

n: number. UAP: unstable angina pectoris. AMI: acute myocardial infarction. TIA: transient ischaemic attack. ATE: arterial thromboembolism. SD: standard deviation. LDL: low density lipids. HDL: High density lipids.

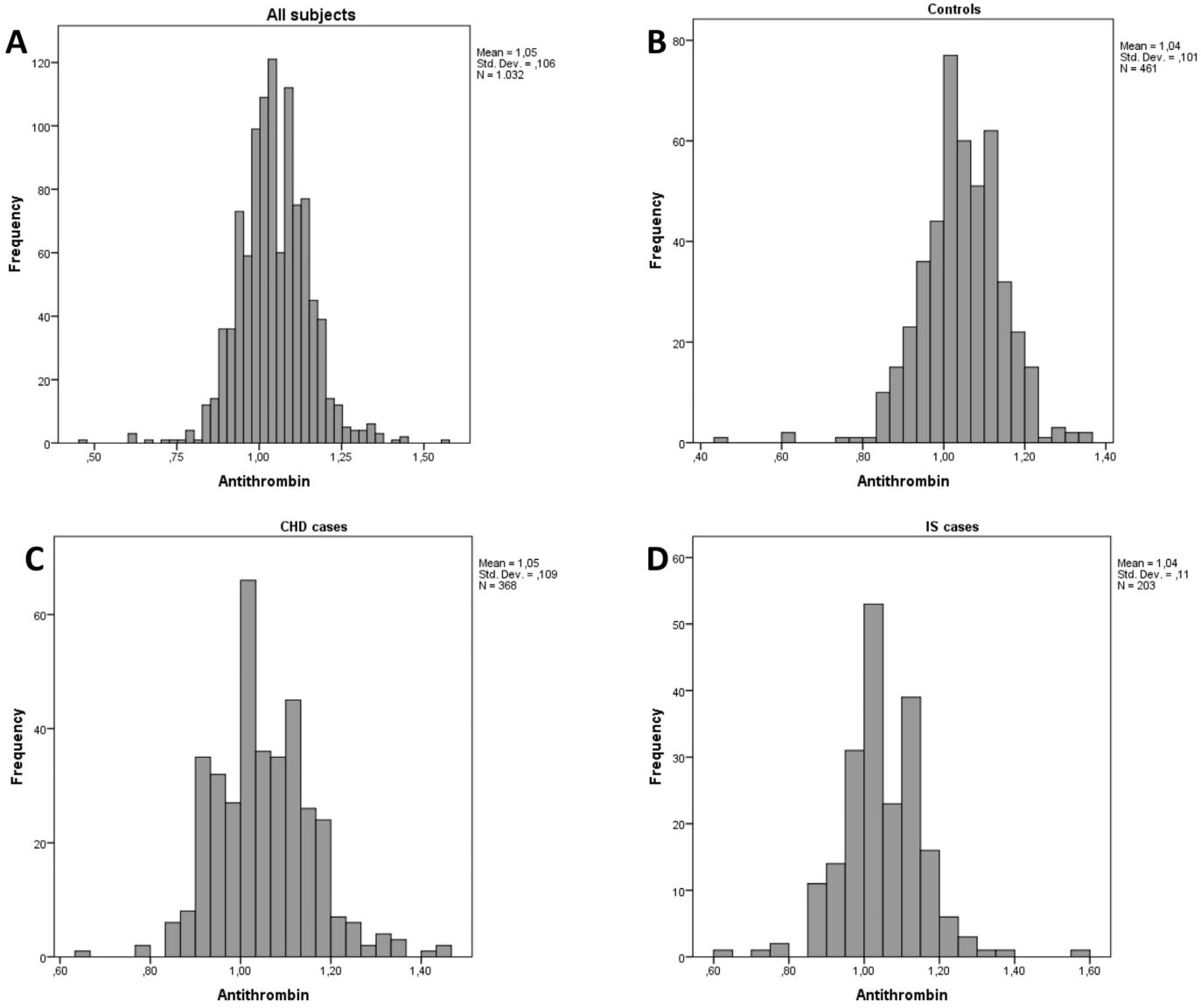


Fig. 1. Distribution of antithrombin levels across categories in the ATTAC study.
Distributions of antithrombin levels in all subjects (A), controls (B), coronary heart disease (CHD) patients (C) and in ischaemic stroke (IS) patients (D).

Table 2
Antithrombin as risk factor for a first arterial thromboembolism in the ATTAC case-control study.

	CHD + IS	CHD	IS
Antithrombin continuous			
Unadjusted	1.53; 0.48–4.89		
Adjusted[a]	0.46; 0.12–1.85	0.44; 0.09–2.28	0.58; 0.09–3.68
Antithrombin median or lower (≤1.04 U/mL)			
Unadjusted	1.12; 0.87–1.43		
Adjusted[a]	**1.46; 1.09–1.96**	**1.43; 1.01–2.02**	**1.48; 1.01–2.19**
Men, adjusted[a]	1.38; 0.86–2.24	1.27; 0.76–2.13	1.97; 0.92–4.20
Women, adjusted[a]	**1.46; 1.00–2.14**	1.56; 0.97–2.52	1.33; 0.84–2.12
Subjects aged <46, adjusted[a]	**1.55; 1.09–2.21**		
Antithrombin deficiency (≤0.80 U/mL)			
Unadjusted	1.21; 0.34–4.33		
Adjusted[a]	1.52; 0.33–7.01	1.42; 0.19–10.54	2.06; 0.36–11.84

CHD: coronary heart disease. IS: ischemic stroke. Bold: statistically significant effect.
[a] Adjusted for all cardiovascular risk factors.

Of all patients, 99% used anti-platelet therapy, 94% used blood-pressure lowering drugs and 95% used statins. Median follow-up time was 3.6 years (range 0.05–9.36 years). Nine patients (3%) died and 37 (11%) had a recurrent arterial thrombotic event.

Twenty-eight of the 181 men (15%) and 20 of the 142 women (14%) had a recurrent event or death. In the total group age, sex and BMI did not differ between patients with or without a recurrent event. Of the 142 women, 45 were postmenopausal, 97 were not. In postmenopausal women antithrombin levels were higher than in premenopausal women mean in premenopausal women 1.01U/ml ±0.10, in postmenopausal women 1.07 ± 0.11 ($p = 0.008$).

Classical cardiovascular risk factors were not associated with recurrent events. A sex-specific analysis showed that diabetes was associated with recurrent events or death in men (HR 3.74, 95% CI 1.15–12.12). In women, none of the classical cardiovascular risk factors were associated with recurrence. Furthermore, menopausal state or use of oral contraceptives was not associated with recurrence. For a summary, see Table 3.

The median antithrombin level was 1.04 ± 0.11 U/ml in the total CHD cohort, 1.05 ± 0.11 U/ml in men and 1.02 ± 0.11 U/ml in women (men *vs.* women, $p = 0.003$).

In the total group of CHD patients, Kaplan-Meier analysis showed that patients with lower antithrombin levels, predefined as ≤ median level of the cohort (≤1.04 U/mL), had a significantly higher risk of recurrent cardiac events ($p = 0.03$). When stratified by sex, this was observed only in women ($p = 0.021$) but not in men ($p = 0.88$) (Fig. 2). Regarding recurrent ATE and death, a higher risk of events was found for participants with lower antithrombin levels (predefined as ≤ median), but this was not significant ($p = 0.12$). When stratified by sex, women with lower antithrombin levels had a significantly higher risk of events ($p = 0.012$) than women with higher antithrombin levels, while no difference was seen in recurrent ATE or death between higher or lower antithrombin in men ($p = 0.82$).

In the total group of CHD patients, patients with lower (≤median) antithrombin levels had a higher chance of a recurrent cardiac event, with a HR of 2.11, 95% CI 1.06–4.21; after adjustment for age, BMI and sex: HR 2.12 95% CI 1.06–4.26; and adjustment for all risk factors: HR 2.16, 95% CI:1.07–4.38. Women with lower antithrombin levels had a higher rate of recurrent cardiac events: HR 6.01, 95% CI 1.38–26.24; after adjusting for age and BMI: HR 6.08, 95% CI 1.38–26.84; after adjusting for all risk factors: HR 5.97, 95% CI 1.31–27.13. In men, after adjusting for all risk factors no significant difference was found, HR 1.18, 95% CI 0.46–3.03.

For recurrent ATE and death in all CHD patients, no significantly different event free survival was observed for participants with lower antithrombin (adjusted for all factors: HR 1.67, 95% CI 0.92–3.02). After stratifying by sex, only women with lower antithrombin levels had a higher risk of recurrent ATE or death, with a HR of 4.2 (95% CI 1.25–14.69); after adjustment for age and BMI: HR 4.38 (95% CI 1.26–15.17); after adjustment for all cardiovascular factors: HR 4.22 (95% CI 1.19–15.00).

4. Discussion

The main finding of the case-control study is that reduced antithrombin levels (≤median) are associated with an increased risk for arterial thrombosis at a young age (OR 1.46; 95% CI:1.09–1.96) when compared with individuals with high-normal (>median) antithrombin levels, after adjusting for major cardiovascular risk factors. This effect is most pronounced in women. In addition, low antithrombin levels are also associated with recurrent events in young individuals with CHD (HR 2.16, 95% CI:1.07–4.38).

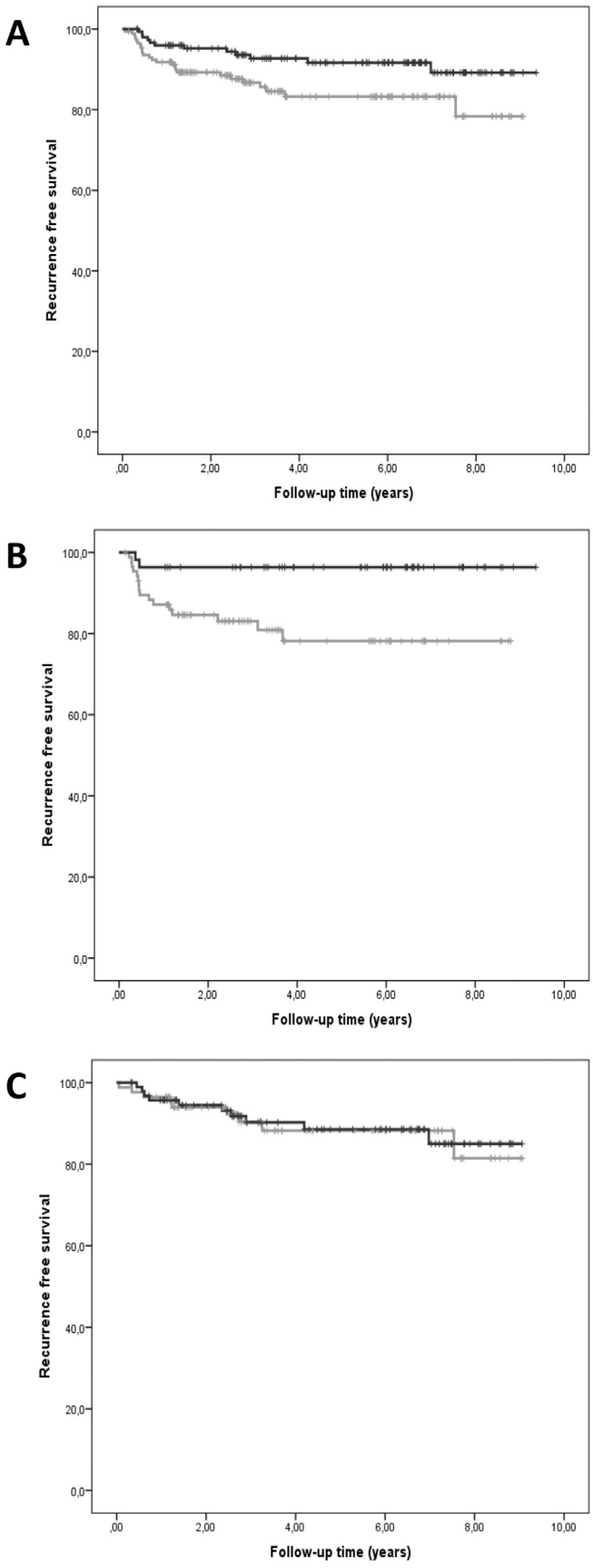
Antithrombin deficiency is the first reported cause of inherited thrombophilia [12], and it is a rare but strong risk factor for venous thromboembolism (VTE). The risk of first VTE is increased up to 16 folds [13]. Furthermore, an inverse relationship between the risk of VTE and antithrombin levels within the normal range has been described; as compared with individuals with high-normal antithrombin levels, in individuals with antithrombin levels in the lower-normal range, the risk of VTE is significantly increased [14]. Although venous and arterial thromboembolism are believed to be different disease entities, risk factors overlap. Inherited thrombophilia is not only associated with VTE, but also with an 1.7-fold increased risk of arterial thromboembolism (ATE), as shown in a combined analysis of four studies on thrombophilic families [3].

The finding that ≤ median antithrombin levels are associated with an increased risk for arterial thrombosis at a young age is in part in line with previous studies. These studies focused on inherited antithrombin deficiency and thus on much lower antithrombin levels (usually ≤0.80 U/ml). In a large prospective study in families with thrombophilia, an up to 9-fold increased risk of ATE in individuals with inherited thrombophilia was observed [2]. In this study, antithrombin deficient individuals had a 7-fold increased risk of ATE. More recently, a pooled analysis of four family studies by Mahmoodi et al. showed that inherited thrombophilia overall is a risk factor for ATE [3]. This risk was even more pronounced in individuals below the age of 55 and in women. However, antithrombin deficiency by itself failed to show an association (HR 1.36, 95% CI 0.55–3.34), possibly due to a lack of power, since only 72 antithrombin deficient participants were included in this analysis. Antithrombin levels were not associated with the incidence of ischemic stroke and coronary heart disease in the ARIC study, a large, prospective population study. Of note, the individuals included in this study were older (age at inclusion 45–64) than in our study population, which included males aged ≤45 and females aged ≤55 years [15,16]. In the prospective Northwick Park Heart Study, antithrombin levels and the

Table 3
The risk of recurrent arterial events or death in the follow-up study.
Traditional cardiovascular risk factors, median antithrombin and sex. Cox regression analysis to assess the relationship between traditional cardiovascular risk factors and median antithrombin levels and the risk of recurrent arterial thrombotic events or death (all-cause). None of the 19 women with diabetes had an event.

	Any recurrent event HR (95% CI)	Men HR (95% CI)	Women HR (95% CI)
Age	0.97 (0.92–1.03)	0.95 (0.87–1.03)	1.01 (0.94–1.10)
Female sex	1.05 (0.51–2.17)		
Family history of cardiovascular disease	1.04 (0.58–1.88)	0.79 (0.35–1.80)	1.33 (0.53–3.36)
Hypertension	0.78 (0.36–1.67)	0.83 (0.26–2.67)	0.96 (0.33–2.83)
Diabetes	1.02 (0.35–2.96)	3.74 (1.15–12.12)	
Smoking, current	0.92 (0.41–2.09)	0.78 (0.23–2.58)	0.36 (0.05–2.46)
Smoking, former	0.62 (0.24–1.58)	0.52 (0.17–1.56)	1.83 (0.46–7.29)
Total cholesterol	1.25 (0.88–1.78)	1.30 (0.82–2.04)	0.85 (0.43–1.68)
HDL	1.10 (0.45–2.67)	0.84 (0.23–3.06)	2.58 (0.69–9.66)
BMI	0.97 (0.90–1.03)	0.93 (0.84–1.03)	1.01 (0.92–1.12)
Median antithrombin (≤1.04 U/mL)	1.67 (0.92–3.02)	1.06 (0.48–2.35)	4.22 (1.19–22.56)

HR: hazard ratio. HDL: high density lipids. BMI: body mass index.



association with ischemic heart disease were investigated in men only, aged 40–64. In this study, individuals with antithrombin levels in the lowest tertile had a higher risk of death from arterial disease when compared to the middle tertile [17]. However, this was also observed for the highest tertile as compared to the middle tertile. The increased risk of ATE associated with low antithrombin levels, in our large patient population with a first manifestation of ATE at an even younger age, is therefore a novel and interesting finding.

In order to investigate whether antithrombin levels are associated with the risk of recurrent ATE, we subsequently followed 323 patients with CHD from our case-control study, during a median of 3.6 years, for recurrent events. The main finding is that lower antithrombin levels were associated with a higher risk of recurrence, with a HR of 2.16, 95% CI: 1.07–4.38. The main contribution to the increased risk comes from women with lower (≤median) antithrombin levels. Women with lower antithrombin levels did not only have a high rate of recurrent cardiac events (HR 5.97, 95% CI 1.31–27.13), but also of recurrent ATE and death (HR 4.22, 95% CI 1.19–15.00) as compared to women with higher antithrombin levels. For men, having a ≤median antithrombin level was not a risk factor. This sex-specificity of lower antithrombin levels on recurrence risk of CHD has not been reported before, but gender specificity for thrombophilia as a risk factor for a first ATE has been reported before [3]. In IS patients, familial thrombophilia has been reported as risk factor for recurrent ATE, but antithrombin levels were not investigated [18]. Only one study has previously investigated the role of antithrombin levels on recurrent ATE in CHD patients. In a prospective cohort study by Pelkonen et al. on acute coronary syndrome patients, patients who had a recurrent arterial event had lower antithrombin levels than patients who had no recurrence [19]. However, these subjects were mostly male, and substantially older (64 ± 10 years) than in our study (43.6 ± 5.8 years). No sex-specific analysis was performed in this study, and therefore it is difficult to compare the results. The pathogenetic explanation of how lower antithrombin levels increase recurrence risk only in women has not yet been resolved. Many other gender differences in (risk factors of) coronary heart disease have been reported before [20].

Current concepts of the pathways involved in thrombus formation in acute coronary syndrome include both platelet activation and fibrin formation [21]. The latter pathway is controlled by antithrombin [1]. The hypothesis that lower antithrombin levels would increase the risk of (recurrent) cardiac events by tilting the hemostatic balance towards a more prothrombotic phenotype seems logical. When patients present with ATE, many of the risk factors causing the first arterial event such as hypercholesterolemia, diabetes, hypertension and possibly factors related to primary hemostasis will be actively managed by lifestyle adjustments or medication, as in our cohort. Therefore, other possible risk factors pertaining to inhibition of thrombin formation may become more apparent. Through these risk factors high-risk patients may be identified and potentially treated differently.

Our studies have limitations. The chosen definition of hypercholesterolemia at inclusion in the study, which includes the use of statins, irrespective of the reason why they were prescribed, did not allow for an adequate analysis of this in the case-control study. The different age-limit for men and women for inclusion in the study could have an effect on results, however, when excluding subjects

Fig. 2. Recurrent cardiac event-free survival according to median antithrombin levels. (A) Recurrent cardiac event-free survival overall. (B) Recurrent cardiac event-free survival in women. (C) Recurrent cardiac event-free survival in men. Black bars: antithrombin level > median (1.04 U/mL); grey bars: antithrombin level ≤ median (1.04 U/mL).

(women) of age 46 or older, the effect of low antithrombin levels in the case-control-study was more pronounced, HR 1.55; 95% CI 1.09–2.21. The blood samples may have been influenced by post-event changes such as coagulation activation [22,23]. This was, however, not tested. Therefore, the association of low antithrombin levels and ATE can result from the ATE itself through coagulation activation, or a low antithrombin levels may be a contributing cause of ATE. Post-event inflammation is unlikely to have played a role as hsCRP levels were low. At inclusion in the study and drawing of blood, 95% of patients used platelet function inhibitors and 8% vitamin K antagonists. Previous studies have shown that vitamin K antagonists (warfarin) do not change antithrombin levels [24]. We are unaware of studies showing that platelet inhibiting drugs (clopidogrel, aspirin) influence antithrombin levels. None of the subjects used (low-molecular-weight-) heparin at the time of blood sampling.

Although our follow-up study of young patients with a first cardiac event is quite large for these type of patients, the number of recurrent events was still low. This limits the statistical power to detect risk factors. Therefore, a rather crude cut-off of median antithrombin levels was chosen to investigate if there was any effect. If on a population level a more narrow cut-off is more predictive is unknown. This limits the use of antithrombin levels as a predictive tool in practice based on this study alone. The low statistical power may also explain that, of the classical cardiovascular risk factors, only diabetes in men was a significant risk factor for recurrence, while other classical cardiovascular risk factors were not. Even so, risk factors for first and recurrent events are not necessarily the same [25]. Despite this, the finding of a high risk in women with low antithrombin levels for ATE and recurrent ATE seems to be robust and in line with previous studies [3], but should be validated in further studies. Another limitation of the follow-up study is the lack of information on adherence to medications prescribed, as non-adherence has been shown to be an important risk factor for recurrent ATE [26].

In conclusion, we found that lower antithrombin levels increase the risk of a first arterial thrombotic event at a young age. Lower antithrombin levels are associated with a higher rate of recurrent cardiac events and with a higher rate of recurrent arterial thromboembolism or death in women.

Conflicts of interest

The authors declare no conflict of interest regarding this research. F.W.G. Leebeek is consultant for UniQure and Shire (fees go to the institution), received unrestricted research grants of CSL Behring and Baxalta (Shire), and travel support from Roche, all outside the submitted work.

Financial support

This work was supported by a MRACE grant (2006) of the Erasmus MC, Rotterdam, the Netherlands (F.W.G. Leebeek).

Author contributions

F.N. Croles performed analyses and wrote the manuscript. J.E. Van Loon performed analyses and critically revised the manuscript. D.W.J. Dippel designed the study and critically revised the manuscript. M.P.M. De Maat designed the study and critically revised the manuscript. F.W.G. Leebeek designed the study and wrote the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.01.014.

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